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DETECTION OF EXPLOSIVE VAPORS BY ENZYMATIC METHODS.(U)  
MAY 79 A STRICKLER, C BRAUNER

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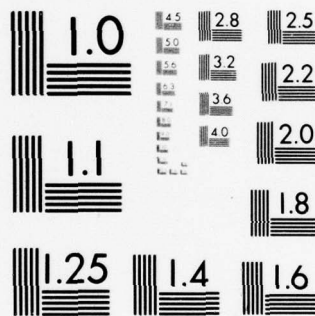


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DETECTION OF EXPLOSIVE VAPORS  
BY ENZYMATIC METHODS.

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A. Strickler and C. Brawner

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Contract No. DAAK 70-78-C-0074

11 May 1979

12 24p.

DDC FILE COPY

Prepared for:

U.S. Army Mobility Equipment  
Research and Development Center  
Ft. Belvoir, VA 22061

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## 1.0 INTRODUCTION

The present report documents a continuation of the effort last described in the report of January, 1978, Contract No. DAAK02-74-C-0100.

At the end of the prior contract period, an experimental continuous flow system had been built, and preliminary tests made on its performance. Improvement was evidently necessary in several areas: flow control of the reagent and contactor liquid streams, design of the phase separator, and design of the detector assembly for elimination of stray light. A simple vapor contactor had been built and tested for efficiency. However, it was not yet known whether it was of optimum design in view of the pertinent literature and work of other investigators.

The objectives of the present effort, then, were to: a) survey the state of the art in vapor/aqueous system contactors, b) select, build, and test an appropriate contactor unit, c) design and test an improved continuous flow reaction system, d) integrate the contactor and reaction/detection system, and e) test performance of the combined system over a range of TNT-in-air concentrations.

## 2.0 STUDY OF VAPOR-LIQUID CONTACTORS

### 2.1 State of the Art in Contactor Design

The design and performance of the contactor are critical for several reasons. Most important, the efficiency of transfer of TNT vapor into the liquid phase must be high to avoid a serious loss of sensitivity. A relatively large volume of air sample, of the order of several liters to several tens of liter, must contact a relatively small volume of liquid, about 1 ml, each minute. Further, the contactor must not significantly increase the response time of the system. Specifically, its washout time should not be long compared with the chemical response time of the system. Trace amounts of explosive vapor must not be irreversibly



adsorbed on the contactor surfaces. The contactor must also be reliable, have a low maintenance requirement, and preferably be a simple design suitable for use in a mobile or portable unit.

There exists an extensive technology relating to contactors of various types. Unfortunately, little of it is directly applicable to the present problem. The systems for contacting large air volumes with small liquid streams were designed mostly for particle collection. Such systems make use of particle inertia effects, and as such are generally inefficient for trapping traces of vapor. In contactors designed specifically for gas analysis, the volume ratio of gas to liquid has not usually been very high, nor the washout time as rapid as we require. Gas scrubbers used in chemical processing and pollution control equipment have similarly required neither very large volume ratios nor rapid washout. The technique of concentrating the trace vapor on an adsorbant (for example charcoal or molecular sieve), as practiced for example in gas chromatography, is inapplicable for our needs because small traces of vapor, at the levels we must detect, are nearly impossible to desorb (for subsequent detection), even at elevated temperatures.

A mist-coalescence type of contactor, such as built some years ago by Cambridge Technology, Inc., for Edgewood Arsenal, would appear to be an attractive approach for trace vapor detection. Unfortunately, this system was found to be only 15 to 20% efficient for vapor collection, although much better for collection of microscopic particles. Further difficulties are that the mist-forming mechanism often creates a maintenance problem, and deposition of mist on the contactor surfaces may result in a long washout time.

After evaluation of the approaches shown in the literature, and consultation with personnel at Chemical Systems Laboratories (Edgewood), Southern Research Institute, and Beckman Instruments, Inc., a simple design was adopted of a type reported by Goodson and Jacobs,<sup>1</sup> and used also by the Beckman Process Instruments Division in their "Acralyzer"

oxidant detector systems. This comprises a straight glass tube into which is closely fitted a helical element that increases the contact surface area.

## 2.2 Fabrication and Test of Helical-Element Contactors

Two different types of helical contactor were fabricated for us by a vendor. In one type, a close-fitting rod, machined with a helical groove in its surface, was inserted into a glass tube of 8-mm ID. The groove was approximately of rectangular cross section, about 4 mm wide and 1.5 mm deep. In the other design, shown in Figure 1, a close-fitting helical coil made from 1/16-inch-diameter glass rod was fitted into a tube of 8-mm ID, with a turn spacing of 6 mm.

The contactor with the machined internal rod was set aside early in the experiments because it proved to have too narrow a channel and excessive resistance to air flow. Tests were made on the contactors with various liquid and air flow rates, and with flow in both co-current (upward and downward) and counter-current mode. These experiments and later experience indicated that flow behavior was best with the helical-coil contactor, using downward co-current flow. A disadvantage of upward liquid flow was that pooling of liquid would occur in the column even at high (20 l/min) air flow. In downward flow, this effect was absent over a wide flow range. The minimum practical liquid flow rate was about 1 ml per minute, with air flow up to about 4 liters per minute. At higher air flows, pressure drop in the contactor tube became excessive and started to present a problem in maintaining liquid flow through the system after the separator. However, if, as we suggest later, a peristaltic pump is applied to the outlet end of the flow system, it should be possible to use higher air flows.

The washout time was determined by flowing HCl solution through the contactor at about pH 3, and abruptly changing the input to distilled water while monitoring the pH of the effluent. The washout time was 3 or 4 minutes for a pH change of 3 to 4. This represents a decade change in

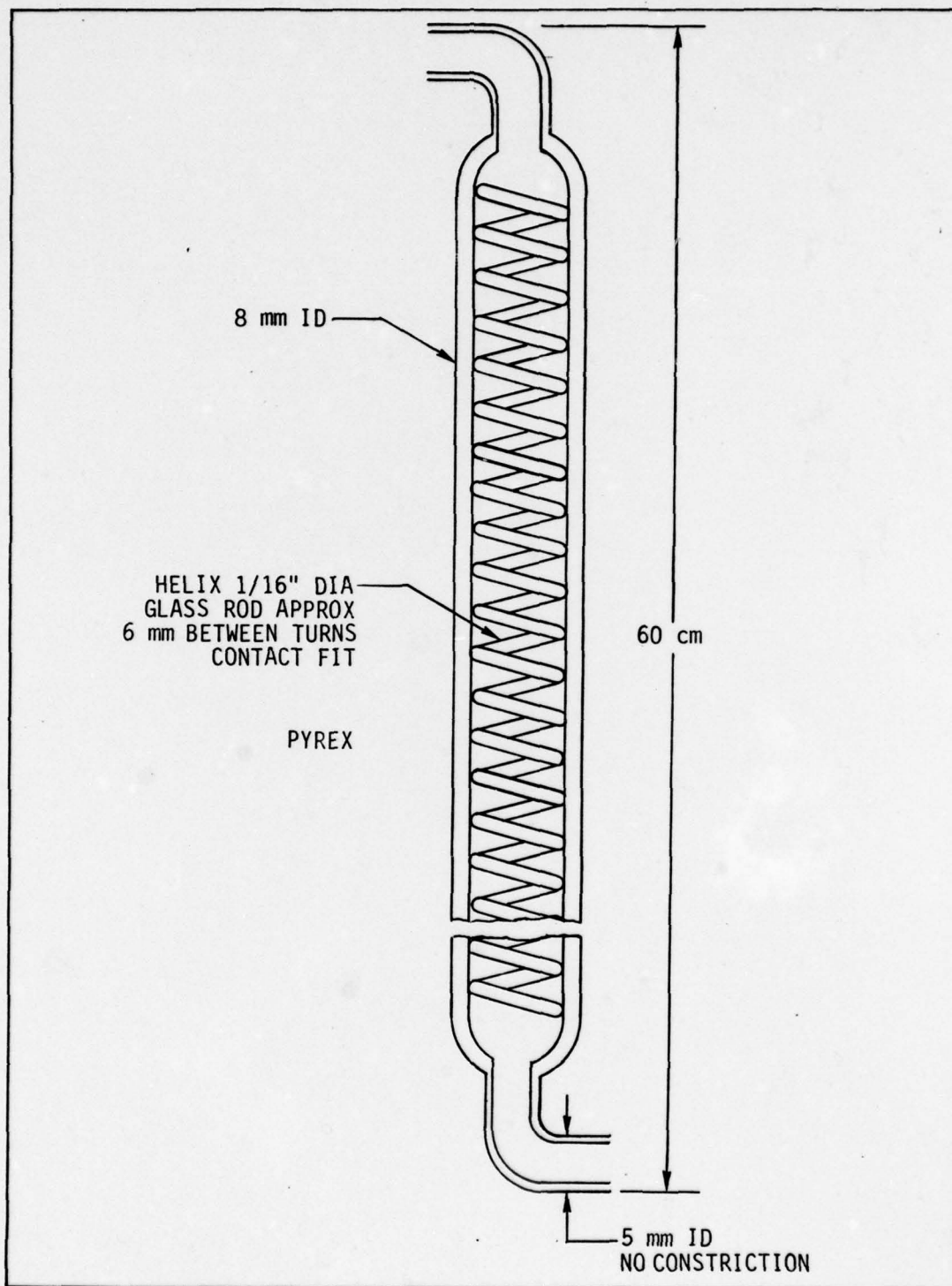


Figure 1. Experimental Contactor (Helical Coil Model)



concentration or about 90% washout. Although a washout time of less than a minute would be preferred, the contactor appeared satisfactory for the present preliminary experiments.

### 3.0 CONTINUOUS FLOW SYSTEM: EXPERIMENTAL STUDIES

#### 3.1 System Concept

A block diagram of the experimental system is shown in Figure 2. Entering the top of the contactor is the air sample stream and a liquid stream of NADH-containing buffer. Pump 1, feeding this liquid stream, is of peristaltic type similar to pump 2. The air pump, drawing in the air sample via the bottom of the contactor and the phase separator, is of the vibrating diaphragm type.

The liquid effluent from the phase separator joins a stream of TNTase solution in mixer 1. Reaction of the NADH with TNT begins in this mixer and continues in the delay element. The latter is designed to allow the reaction to continue for 30 seconds to 1 minute (the same as in the bench-top procedure) before the next reagent addition.

The "reaction mix" from the delay element enters mixer 2 where it joins the combined streams of luciferase and tetradecanal. The luciferase reaction is very rapid, probably being complete within several milliseconds once the components are mixed. It is essential, therefore, that all of the mixing take place, if possible, within full view of the photomultiplier. This is done by bringing the reaction mix stream and the combined luciferase/tetradecanal stream into the reaction cell coaxially with respect to each other, with the streams joining at the center of the photospiral and within its main plane adjacent to the photospiral. As we show later, the desired mode of mixing of the stream is by diffusion. This results in a stretch-out of the mixing process, such that chemical reaction and light emission occur over most or all of the spiral path.

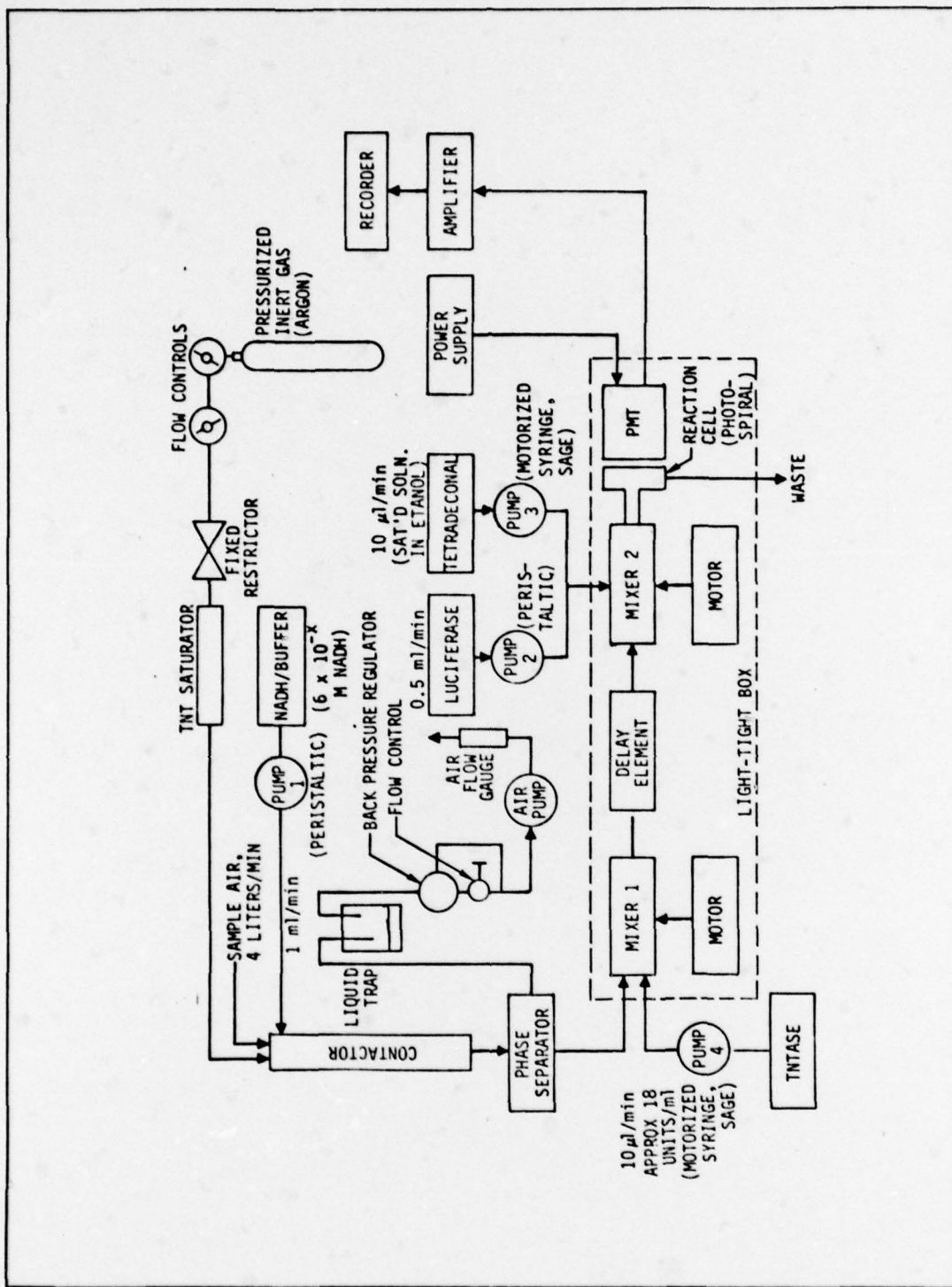


Figure 2. Experimental Continuous System--Schematic

As shown by the dotted line in Figure 2, the mixers, drive motors, delay element, photospiral, and PMT are all enclosed in a light-tight box. Although light traps were initially fabricated for blocking entry of light into the box via the fluid lines, these were later found unnecessary. The opaque black Teflon tubing (about 0.030-in. ID) leading into the box was found by itself to be a sufficient light trap.

Finally, as shown in the figure, the signal from the PMT is amplified by an electrometer amplifier and applied to a recorder.

### 3.2 Components and Subassemblies

The following is a further description of various features of the system:

- Liquid Pumps

Low liquid flow rates, required for the TNTase and tetradecanal solutions, were provided by a Sage motor-driven syringe pump. (Sage Instruments, Division of Orion Corporation, Cambridge, MA 02139). Flow rate in this type of pump can be changed by using various sizes of syringe and incremental change of the motor speed. Relatively high liquid flow rates, as required for the NADH and luciferase, were obtained by using individual, single-channel peristaltic pumps (Markson Science, Inc., Del Mar, CA). The desired feed rate is obtained in this type of pump by choice of tubing diameter and selection of the pump model having nearest to the required rpm.

- TNT Vapor Feed

Two arrangements were built for TNT vapor sampling. In one of these, a regulated flow of inert gas (argon) passes through a tube of TNT-coated Chromosorb G, according to the method of Pella.<sup>2</sup> In the other, the inert gas passes through a vessel in which is contained the TNT permeation tube. The first-mentioned arrangement is preferred, since the rate of TNT release from the permeation tube (a very low value) is unknown, and would be difficult to calibrate by any known method.



- Phase Separator Design and Liquid Flow Path

The phase separator is illustrated in Figure 3. Its large internal diameter, compared with that of the contactor tube, allows the air stream to slow down, preventing carryover of droplets into the air flow regulator.

The principle of the reactor flow arrangement was that the liquid in the system following the phase separator would in effect flow through an upright, U-shaped path. Mixers 1 and 2 plus the reaction cell would lie within the U, which would start at the bottom of the separator and end at an opening or drain point at a certain level lower than the meniscus in the phase separator. The difference in hydrostatic head between the meniscus in the separator and the outlet of the U would correspond to the air pressure drop within the contactor. Our experiments did not proceed far enough within the time available to test carefully the behavior of this part of the flow scheme. Instead, most of our experiments preceded the step at which we would introduce TNT vapor, and therefore bypassed the contactor and phase separator. However, our preliminary tests indicated that this flow arrangement, driven only by a small, gravity-induced pressure differential, would not be reliable. A superior arrangement would consist in active pumping of the reaction stream from the outlet of the photospiral. A sipper arrangement in the phase separator would remove excess flow from the contactor beyond that needed for the reaction stream, and maintain a constant meniscus level in the separator.

- Air Sample Flow Control

A Thomas vibrating diaphragm pump (Thomas Industries, Sheboygan, WI, Model 107CA050) is used to draw the air through the contactor. A liquid trap in the air stream following the phase separator prevents carryover of liquid droplets into the



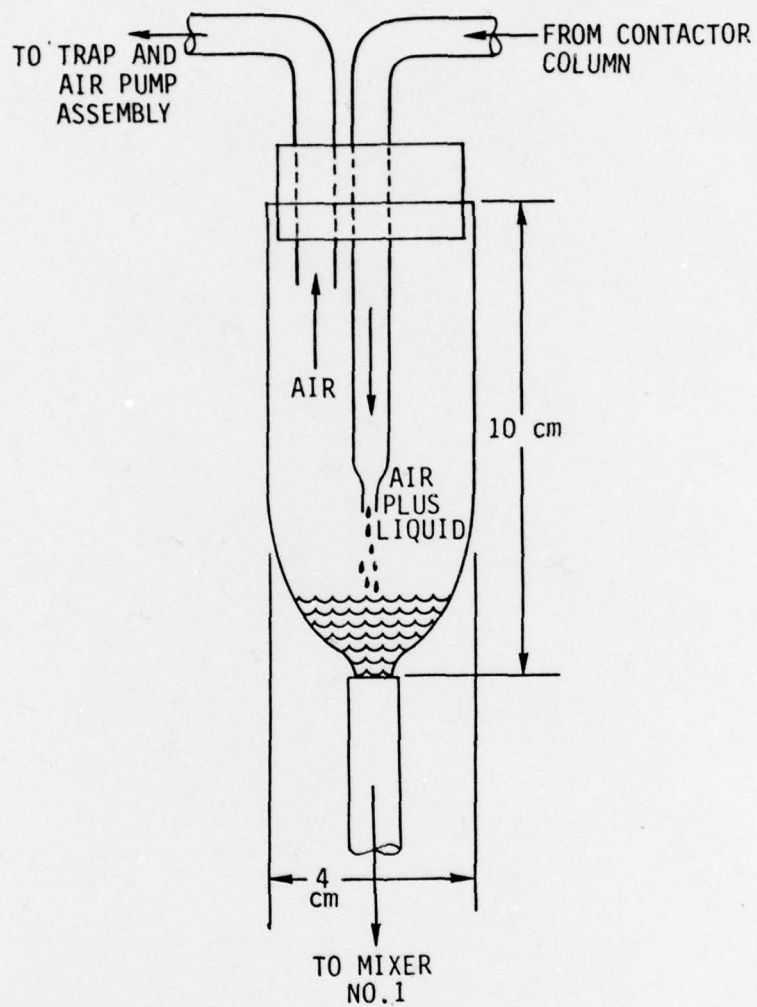


Figure 3. Phase Separator

regulator. The latter is designed to control the pressure at the inlet of the air pump. Adjustment of the needle-valve sets the flow at the desired constant rate.

- Mixing Units and Motors

The designs of mixers 1 and 2 are shown in Figures 4 and 5, respectively. Mixer 1 is a small-volume device with minimum dead space which rapidly mixes the contactor effluent with TNTase solution prior to entering the delay element. The cylinder cavity in this mixer is fitted with a rotating stainless-steel bar, the surface of which is cut with a right-handed groove of rectangular cross section. The cavity itself is cut with a left-handed groove. It was expected that this combination of grooves would minimize pumping effect by the wiper. It developed, however, with our present system which lacks positive pumping at the outlet of the photospiral, that this mixer nevertheless exerted an undesirable pumping action of its own, creating erratic flow. Therefore, for subsequent experiments, the motor of this mixer was turned off and diffusion alone in the mixer and delay line relied upon for mixing, the same as described below for mixer 2.

Mixer 2 introduces the "reaction mix" from the delay element to the center of the photospiral by way of a hollow needle. The luciferase/tetradecanal mixture flows along the outside of the needle to meet the reaction mix in the photospiral. A motor rotates the needle, which is shaped at its outer end to cause mixing of the two streams. As discussed later, this mixing action turned out to present a problem, in that agitation of the luciferase/tetradecanal, even in the absence of any NADH, generated light to produce a large signal. Subsequent experiments therefore were done with the motor of mixer 2 turned off, and diffusion alone allowed to effect the mixing.

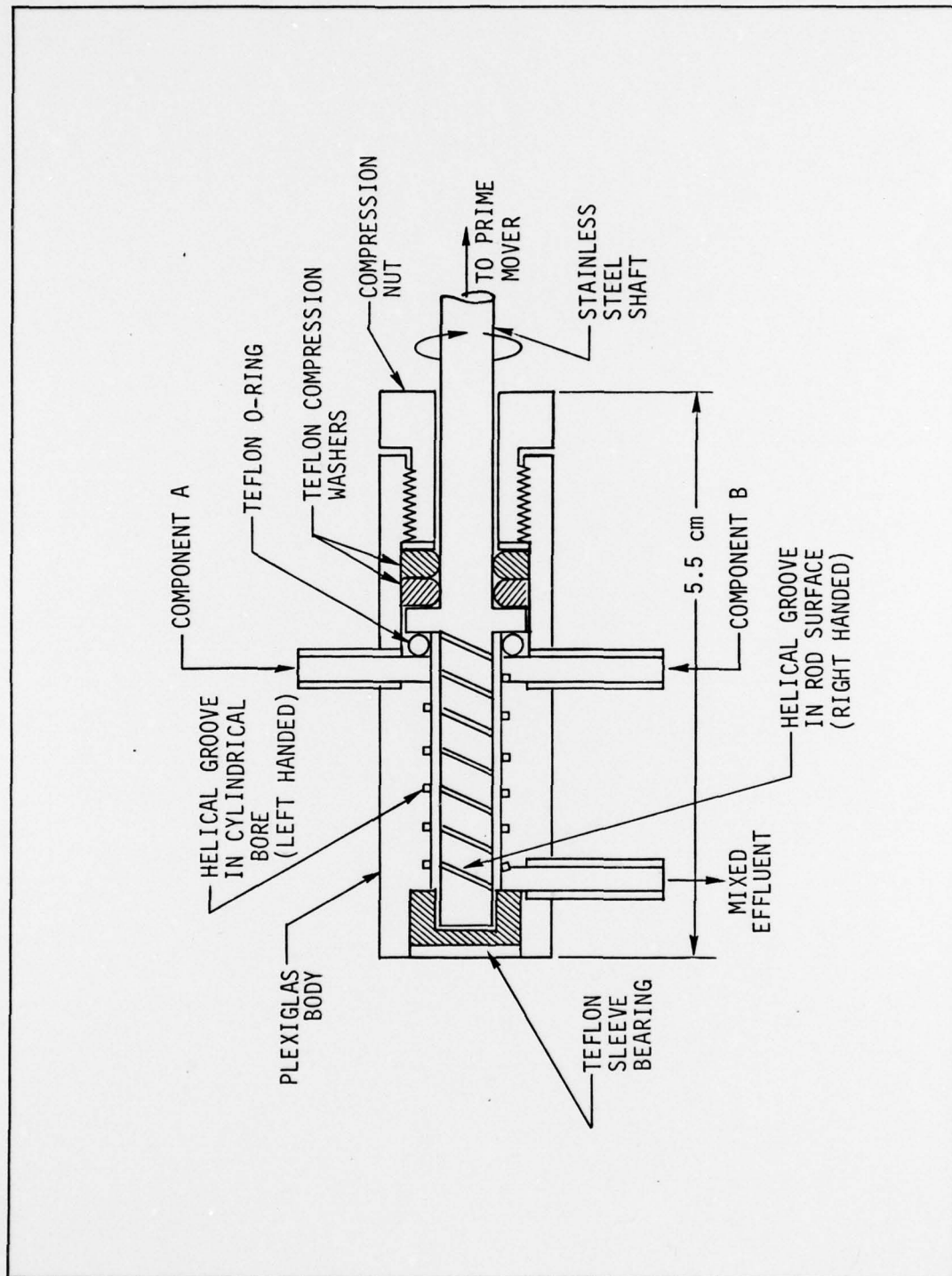


Figure 4. Mixer No. 1

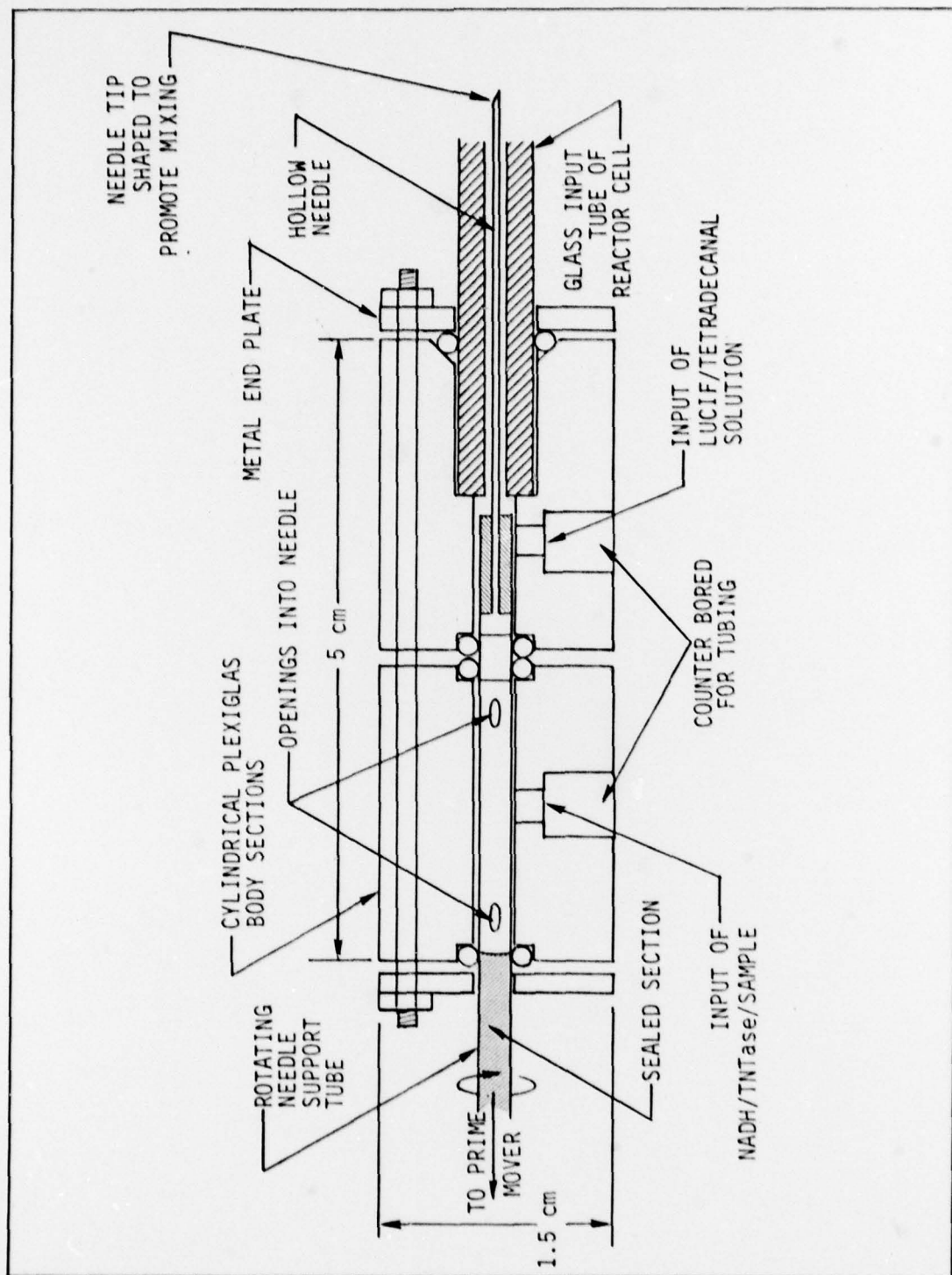


Figure 5. Mixer No. 2



The motors for both mixers were of miniature dc type, about 1-1/4 inch long, manufactured by Portescat (Switzerland). There was no apparent interference by these motors (due to electrostatic or magnetic fields) with the photomultiplier signal.

- Chemiluminescence Reaction Cell (Photospiral)

As described in the Technical Report of January, 1978, the chemiluminescence reaction cell is a spiral of capillary tubing, about 17 cm long and 1 mm in ID. It is mounted to fit light-tight against the PMT housing. However, light could enter the ends of the spiral if this were not prevented. The side of the spiral facing away from the PMT is aluminized to increase the efficiency of light collection.

- Photomultiplier and Electronics

The PMT is a Centronic, Type 4283, of broad spectral sensitivity and very low dark current. Typical sensitivity is 200 amperes/lumen, with a dark current of 1 nanoampere, and a dark current-equivalent light flux of  $5 \times 10^{-12}$  lumen. The photon rate at this dark current level is  $4.1 \times 10^4$  photons/s, assuming a mean wavelength of 5550 angstroms. This derives from the relations

$$1 \text{ lumen (5550 angstroms)} = 0.00148 \text{ watt}$$

$$1 \text{ watt} = 1 \times 10^7 \text{ erg/s}$$

$$\nu = (3 \times 10^{10} \text{ cm/s}) / (5.55 \times 10^{-5} \text{ cm}) \text{ s}^{-1}$$

and the Planck equation

$$E = h\nu$$

where E is the energy of a single photon, and h is Planck's constant, equal to  $3.32 \times 10^{-27}$  erg s.

From these relations we have for the proton rate

$$R_p = \frac{5 \times 10^{-12} \times 1.48 \times 10^{-3} \times 10^7}{3.32 \times 10^{-27} \times (3 \times 10^{10} / 5.55 \times 10^{-5})}$$

or

$$R_p = 4.13 \times 10^4 \text{ photons/s}$$

Applied dynode voltage in our experiments was 1000 to 1200 volts, supplied by a regulated high-voltage dc power supply. The amplifier was a Boonton Model 95A Sensitive DC Meter; the recorder a Beckman 10-inch unit.

### 3.3 Experimental Results

The major effort in this contract period was devoted to designing, procuring, assembling, and pre-testing the system elements described above. Only a limited time therefore remained for testing the operation of the system. Nevertheless, we were able to make a preliminary systematic analysis of most aspects of the system's performance.

The photomultiplier dark current throughout our experiments proved to be negligible compared with all other signal levels to be measured, including the luciferase/tetradecanal glow level. The latter was about  $6 \times 10^{-3}$  amperes using the luciferase and tetradecanal flow rates shown in Figure 1. In our earliest experiments on the system, a spurious and much higher glow level was observed. Only later was it found that this was caused by the agitation of the luciferase/tetradecanal by the rotating needle of mixer 2. This spurious level, about  $2 \times 10^{-7}$  amperes, was large enough to obscure the effect of any subsequently added NADH.

With the motor of mixer 2 turned off, and with NADH/buffer mixture added at the rate and concentration shown in Figure 1, the signal level was about 4 times the glow level. (Note that in this experiment, the NADH was pumped directly into mixer 1, thus bypassing the contactor and phase separator). This ratio of NADH signal to glow level signal, in the

absence of TNTase or TNTase/TNT "reaction mix," was abnormally low in comparison with that seen in the bench top method, by a factor of about 4. To determine the reason for this, NADH taken directly from the reservoir was tested for activity in the conventional bench-top procedure, and the response compared with that obtained using NADH which had passed through the peristaltic pump. This indicated clearly that about 0.75 of the NADH activity was being lost in passing through the pump, presumably due to some effect of the silicone rubber tubing in the pump.

In the brief time remaining before the study had to be concluded, an attempt was made to measure the contactor column efficiency. The technique used was the same as that described in the January, 1978, report, employing the bench-top instrument. Although a reasonable baseline value was obtained, i.e., in respect to signal level seen on introducing the NADH/TNTase reaction mix in the absence of TNT to the luciferase mix, no consistent signal change was observed when TNT (diluted from a solution of known concentration) was included in the reaction mix. Such behavior has not been unusual; however, there was no further time in the present instance to investigate this effect further.

#### 4.0 APPROACHES TO SYSTEM DESIGN: CONTINUOUS VS DISCRETE INTERMITTENT) ANALYSES

Most of the work described in the present report was predicated on the need to develop a continuous detection system. In any automatic wet chemical analyzer involving, for example, sampling, reagent addition, mixing, and sensing, there is the option in principle of developing instead an intermittent (i.e., discrete) analyzer. In this case the automated system is essentially a robot mimicking the steps of a laboratory procedure in the hands of a live analyst. Sample, reagents, and diluents can be metered into a reaction vessel by motor-driven syringes, valves can be actuated by solenoids, and the reaction vessel intermittently emptied by a pre-programmed sequencer, for example a computer-based controller. However, experience in commercial instrumentation has repeatedly seen intermittent wet chemical systems discarded in favor of



continuous systems. The basic reason is that intermittent systems require many moving parts, often in contact with solutions, and that these are trouble-prone. Continuous systems generally contain far fewer parts and are more reliable. Sensitivity, response time, and repeatability will be at least as good in continuous systems, and possibly better, and standardization is no less convenient.

If one analyzes the bench-top procedure on which the present enzymatic detection scheme is based, it is evident that a corresponding robot analyzer would be mechanically very complex. At least five intermittent metering devices, each with a separate driver, would be required. The system would need mixers, especially since diffusion-mixing is probably not practical in a discontinuous system, and would require a reaction-vessel emptying mechanism. Numerous electromagnetically actuated valves would be needed, posing a significant maintenance problem, particularly where biochemically active solutions are involved. The programming system would in itself be quite complex. In a word, all considerations weigh heavily in favor of the continuous system approach adopted in the present study.

## 5.0 CONCLUSIONS

Significant progress was made during the study. An extensive study of contactors led to a design that appeared reasonable in view of the published literature and experience of other investigators. A practical arrangement was devised for introducing controlled concentrations of TNT vapor. The problem of fluid flow control appeared to be solved for present purposes by the use of peristaltic and motor-driven syringe pumps. A convenient and simple solution was found for the problem of excluding stray light from the chemiluminescence reaction cell. We identified as a significant problem the generation of light by agitation of the luciferase-tetradecanal mix, pointing to the probable need for diffusion-type mixing to produce the chemiluminescence reaction. →



↓  
Various problems remain to be solved, and various improvements made. The effect of NADH oxidase in the TNTase reagent continues to be the most serious single limitation on ultimate sensitivity of the system. Another problem is that it has not yet appeared feasible, in a simple design, to contact the air sample at the required volume rate (several liters to tens of liters per minute) with a smaller liquid stream than about 1 ml per minute. Basically, this may result in a loss of up to 50-fold sensitivity as compared with the bench-top method.

Several problems of instrument design remain:

The photospiral as presently dimensioned was not intended as a diffusion-mixing chamber. For such use, the transit time (now about 5 seconds) is probably too short by a factor of 5 to 10 to permit complete diffusion mixing.

The system should be redesigned to provide pumped flow, rather than gravity flow, from the phase separator through the reaction path and photospiral.

We have mentioned the problem of an apparent destructive effect by the peristaltic pump tubing on the NADH activity. This may perhaps be eliminated by using a different tubing composition, or by using a pump of other than peristaltic type.

It would be very desirable to reduce substantially the washout time of the contactor to, say, 30 seconds, but this would no doubt be difficult.

We note that the efficiency of the present contactor design for TNT trace vapor collection has yet to be determined, although similar designs (with different vapors) have shown reasonable efficiency in the hands of other investigators.

In weighing the desirability of continuing development of the present enzymatic method, several factors need to be considered. The NADH-oxidase problem seriously limits the available sensitivity and practicality of the method. Optimistically, the present limiting detectability is of the order of  $10^{-14}$  mol TNT vapor. Elimination of the oxidase effect could improve the sensitivity by perhaps an order of magnitude. In view of the investigations to date, however, the likelihood of eliminating the oxidase effect, even with a concerted and costly effort, is problematical. Aside from this, a major effort would be required to improve contactor performance, which in effect now depresses the sensitivity about 50-fold compared with the bench-top procedure. It would be desirable to collect the TNT vapor contained in a 1-minute sampling of air in about 20  $\mu$ l of contactor liquid instead of 1 ml. This will be difficult, and may not be achievable in a practical device for the intended purpose. The remaining system design problems are considered to be secondary; although not trivial, they will probably yield to a reasonable engineering effort.

In sum, a decision concerning further pursuit of the enzymatic analyzer effort must weigh the cost, the probability of success, and the large development effort still required, against the continuing urgent need for an effective explosives vapor detector.

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